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Response of locust bean tree *Parkia biglobosa* (Jacq) Benth. explant to growth promoters for micropropagation

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Abstract

***Parkia biglobosa*, a leguminous savanna species, is a threatened species in some West African countries especially Nigeria, due to deforestation and unsustainable exploitation. To conserve this economically important genetic resource, attempt on its micropropagation was carried out using nodal cuttings of the young stem as the explants. The explants were thoroughly surface sterilized, disinfected and cultured in Murashige and Skoog (MS) basal medium. About three weeks after inoculation, 95% sprouting was recorded. The new spouts were subcultured into MS medium fortified with varied concentrations (0.00, 0.25, 0.50, 0.75, and 1.00 mg/L) of benzylaminopurine (BAP) and indole-3-butyric acid (IBA) and then transferred to the growth chamber at 27±1°C. The 0.75 mg/L of BAP produced highest shoot elongation of 3.60 cm while 1.0 mg/L caused shrinkage of the explants in relation to the control (0.00 mg/L concentration). Attempt to root the plantlets with the above-stated concentrations of IBA led to callogenesis and the callus mass increased with the increasing concentration of IBA.**

Keywords: callus induction, explant, micropropagation, *Parkia biglobosa*

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Introduction

Parkia biglobosa (locust bean tree), is an economically important leguminous species that is indigenous to Africa from where it spread to other regions. The tree is widely distributed on sandy loam soil of Sudan and Guinea savanna areas of Nigeria (Aliero et al., 2001). *P. biglobosa* is a fire tolerant heliophyte which occur in diverse agro-ecological zones, ranging from tropical rainforest with high and well distributed rainfall to arid zones where mean annual rainfall is less than 400 mm. Due to its special adaptive capacity, the species spread across the semi-arid zone of sub-Sahara Africa from Senegal to Sudan (Sacande and Clethro, 2007).

P. biglobosa is a multipurpose fruit tree species with wide range of economic values. Although its chief products are the seeds extracted from pods for condiments in stews and soups, the fruit pulp is also used for soil amendment in agriculture (Booth and Wickens, 1988). Both the tree and the fruit has wide range of utilization in pharmacopeia and traditional medicine. In addition, various parts of the tree have been reportedly used as livestock fodder, fish poison, dye, fuelwood and timber for constructions. Processing of the fruit (locust beans) could offer sizeable investment opportunity for the rural dwellers especially the women along the growing zones (Shao, 2002). As a keenly sought after tree, it is a good candidate for poverty alleviation and food security in savanna zone of Nigeria. Recently, *P. biglobosa* is used in agroforestry practice across the tropics (Nair, 1993), soil amelioration or improvement, shelter belt and to mitigate global warming (Tomilinson et al., 1995).

Despite the great economic potentials of

P. biglobosa, the tree grows in the wild, takes long time (about 10-15 years) to mature. Poor sustainable management and deforestation have affected *P. biglobosa* negatively over the years. In addition, factors such as seed dormancy, bush burning, charcoal making, deforestation and urbanization have seriously depleted the tree population and negatively affected the natural regeneration of this species (Sambe et al., 2010; Sacande and Clethro, 2007). Consequently, the tree is currently being overexploited as there is no serious legal of educational framework in place for its conservation and sustainable utilization. In addition, domestication of the tree is still very rare, despite its contribution to local economy. Etejere et al. (1982) observed that only a small percentage of the seeds produced germinated in the field, leading to low population of the crop in savanna region. Sometimes, low annual mean rainfall, dormancy or infected seeds, bush burning could also contribute to reduce the tree population in the wild (Fasidi et al., 2000).

Micropropagation of trees and crop species is veritable technique for production of quality plants (Murch et al., 2000) and conservation of threatened species (Mutasim et al., 2007) such as *P. biglobosa*. To prevent extinction and derive maximum benefits from the plant, it is necessary to preserve the germplasm, to this end, cell and tissue culture techniques are powerful tools for rapid clonal multiplication as well as for reforestation and tree improvement. Fresh seeds (embryo) of locust bean tree have been used at different time in *in vitro* regeneration of species (Tecklehaimanot et al., 2000). Seed germination had also been carried out using whole seeds of the species, according to Sambe et al. (2010), attempt to propagate through seed may be slow, therefore there is need to extend the propagation using different plant parts as explants.

It is clear, that increasing the production of *P. biglobosa* tree will require regular supply of improved seedlings that will regenerate faster, disease free and drought tolerant. This will help in improving the economy of rural communities through sustainable forest management. Modern propagation method that biotechnology offers will facilitate rapid proliferation of *Parkia* clones. Presently, information on in vitro study of *P. biglobosa* is scanty (Amoo and Ayisire. 2004), callus induction from hypocotyl explants of the species have also been reported (Valentine et al., 2012). There is need to exploit micropropagation of the tree as a mean of mass producing planting material to fast track its domestication and reduce gestation period. The present study therefore assessed the effect of different hormonal concentrations on the micropropagation of *P. biglobosa*.

Materials and Methods

The study was carried out in the tissue culture laboratory, National Centre for Genetic Resource and Biotechnology (NACGRAB), Moor Plantation, Ibadan, Nigeria. The glass and plastic wares used for the study were of high quality, the glasswares were borosilicate Pyrex (Staffordshire, Uk). Chemicals, reagents and hormones used were of analytical/molecular grade purchased from Sigma-Aldrich (St. Louis, USA), BDH (Poole, England), E-Merck (Darmstadt, Germany) and Hi-Media (Mumbai, India).

Sterilization of glasswares and light hand tools

Glasswares were first soaked in chromic acid solution, cleansed with Labolene, rinsed with distilled water and oven dried for overnight at 120 °C before used. While plastic wares (non-sterile) were thoroughly cleansed with detergent, rinsed with distilled water and autoclaved before used. All metallic instruments such as blade holder, forceps scissors etc. were sterilized in autoclave using 15 atmospheric pressure at 120 °C for 30 minutes.

Media Preparation

The medium consist of basic MS composition (Murashige and Skoog, 1962). Preparation of the stock solutions was done as described by Patel (2007). Double distilled deionized water was used to prevent interference of the unknown ions in the water. The basal medium was constituted from the stock solutions. 20 mg of growth regulators Benzyl Amino Purine (BAP) powder dissolved in 2 ml of 1N NaOH made up to 100 ml with distilled water was added into the medium. The pH of the resultant mixture adjusted to 5.8 ± 0.1 using 0.1 N NaOH or 0.1 N HCl, then agar powder was added and the volume made up to 300 ml with double distilled deionized water. 10 ml of the medium was dispensed into each of the culture bottles and sterilized by autoclaving at 120 °C at 15 atmospheric pressure for 20 minutes, cooled at room temperature and kept under 4 °C till use.

Explant preparation and culture inoculation

Explants were sourced from NACGRAB'S screen house from the apices and axillary nodes of a juvenile *Parkia* plants. The length of the explants was about 1.5 cm. The explants were

sterilized as described by Verma et al. (2011) and Oyebanji et al. (2009). Explants were sterilized in laminar flow hood by washing in diluted liquid soap, followed 70% ethyl alcohol for 5 min. The explants were transferred into 20% v/v sodium hypochlorite for 20 min, in 0.1% mercuric chloride for 30 sec and finally washed 4 times with double distilled water.

The explant culture was performed under aseptic condition and standard practice. Explant of single node was inoculated into the prepared MS basal medium. The cultures were maintained at temperature of 27±1 °C and exposed to 12 hours photoperiod regime of 88 μ mol m^{-2} light intensity. After successful induction (12 weeks), the explants were sub-cultured on MS fortified with varied concentrations of cytokinin; benzyl amino-purine (BAP) of 0.00, 0.25, 0.50, 0.75, and 1.00 mg/L and the culture allowed to grow at 27±1 °C and incident light of 101.4μmol. $m^{-2}S^{-1}$. The same procedure was used for the rooting and elongation media except that Indole-3-Butyric Acid (IBA; 0.00, 0.25, 0.50, 0.75, and 1.00 mg/L) was added to the basal MS instead of BAP for rooting medium. All the cultures of different growth promoters' concentrations were in four replicates.

Results

The part of plant used as explants for the micropropagation has effect on shoot elongation. The apices and axillary node explants cultured on MS basal medium only without any hormone showed that elongation occurred faster with shoot apices (Plate 1). The mean shoot elongation of the cultured explant from plant apices was 2.38 cm while the cultured from the axillary buds had mean shoot elongation of 1.32 cm over the same period of time (Table 1). The shoot length of the cultured explants was affected by different concentrations of the growth promoter BAP (Plate 2). Among the concentrations used, 0.75 mg/L of BAP produced highest mean (3.60 cm) shoot elongation *in vitro*, while the least (2.35 cm) was recorded in 1.00 mg/L of the growth promoter application (Table 2) with respect to the explant culture without BAP.

During the course of root induction, the attempt to root the plantlets with varying concentrations of IBA (0.00, 0.25, 0.50, 0.75 and 1.00 mg/L) resulted in callogenesis at the root region and mass of the callus increased with respect to increasing concentration of IBA (Plate 3). The plantlet had the highest callus mass at 1.00 mg/L and least mass at 0.25mg/L but no root was formed (Plate 4).

Plate 1: The response of Apices and Axillary nodes to MS Basal medium

Table 1. The response of apices and axillary nodes to MS Basal Medium.

Replicates	Shoot elongation (cm)	
	Apical explant	Axillary node
1	3.00	1.00
2	2.20	1.80
3	1.50	1.20
4	3.00	1.40
5	2.20	1.20
Mean ()	2.38	1.32

The comparison of the two means using t table showed high significant difference between the apices and axillary nodes at P<0.001,

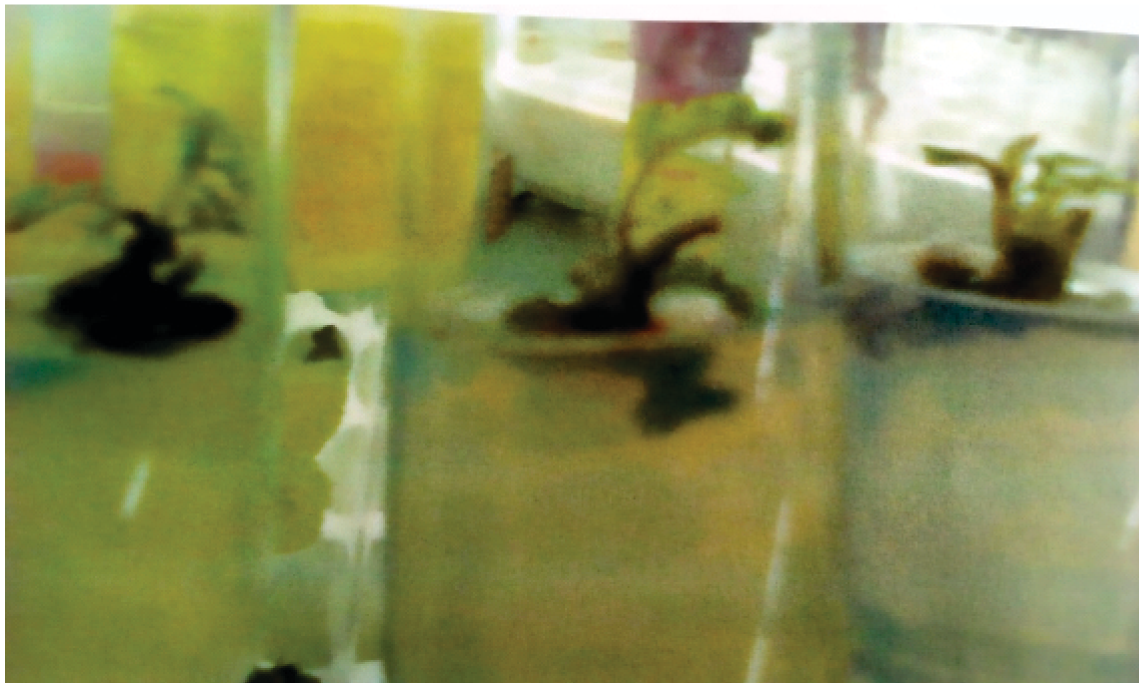

Plate 2. Effects of different concentrations of BAP on shoot length of *in vitro* cultured *Parkia biglobosa*

Table 2. Shoot length (cm) of *Parkia biglobosa* explants after 12 weeks of inoculation with MS fortified with different concentrations of benzyl amino-purine (BAP).

BAP Conc. (Mg/L)	Shoot length/replicates (cm)				Average shoot length (cm)
	1	2	3	4	
0.00	2.60	2.50	2.60	2.80	2.63
0.25	3.30	3.20	2.30	3.20	3.00
0.50	3.20	3.30	3.30	3.60	3.35
0.75	3.50	3.60	3.80	3.50	3.60
1.00	2.30	2.50	2.30	2.30	2.35

Plate 3. Effect of different concentrations of Indole-3-butyric acid (IBA) on rooting of *Parkia biglobosa* plantlets.

Plate 4. Mass of callus as a result of IBA treatment on rooting of *Parkia biglobosa* plantlets

Discussion

The study was carried out to evaluate micropropagating of *P. biglobosa* from apices and auxiliary buds. This is important for the maintenance of genetic information of the mother plant in the micropropagated clones of the *in vitro* propagation of endangered species. The response of apices and axillary nodes to MS basal medium observed in this study. This may be due to actively dividing cells (meristems) that are more concentrated at the apices than all other parts. The current observation was in tandem with Preece et al. (1991), who reported a frequent apical predominance over axillary buds in *in vitro* culture of *Parkia* species. In addition, the present work also corroborates the findings of Ali et al. (2008) on alternative strategy for carnation plant multiplication. While testing the efficiency of type and source of explants, the authors alluded that apices produced more plantlets than auxiliary nodes in microshoot regeneration, thus informing that explant from tip cutting are better for regenerating and elongation of large number plantlets irrespective of the plant species. To further reinforce this, Datta (1992) and recently Jyoti et al. (2014), explained that shoot tips explants and hypocotyls explants are the most suitable in micropropagation of *Dalbergia sisoo*.

The result of the study indicated that basal medium with different concentrations of BAP considerable effect on shoot height of the plantlets. Shoot elongation of the explant in response to different concentration of 0.75 mg/L produced average shoot length of 3.60 cm while concentration 1.00 mg/L caused a drastic reduction in length of the plantlets or shrinkage of the explants relative to the control. This is similar to a report according to Ravishanker (1988), who noticed that higher concentration of BAP above 1.0 mg/L can result in shoot malformation of *Mentha piperata*. The observation supports the well-known inhibitory influence of high concentration of cytokinin on shoot elongation. In a nut shell, depending on the species or cultivar, the most important achievement obtained in the propagation of many plant materials through tissue culture has been frequently based on successful adjustment of type and combination of plant growth regulator (Tran-Thanh Van, 198I).

In the course of root induction, the occurrence of collogenesis in the root region instead of root formation could be attribute to insufficient concentrations of Indole-3-butyric acid (IBA) that were used. Belaizi et al. (1994) alluded that IBA concentrations of 2-5 mg/L promotes rooting and reduced the rate and diameter of callus in *Ceratonia silique*. The authors further explained that IBA concentration of 5 mg/L could prevent formation of basal callus to 0% from the axillary and cotyledonous nodes,

the high concentrations however negatively affected shoot elongation. Consequently, this study suggests that uses of 5 mg/L of IBA for root induction should take effect after the plantlet might have attained their full length that can be acclimatized. This is to prevent the growth inhibition that could be introduced on plantlets by high concentration of IBA. The medium matrix on which the plant was cultured could as well affect the root formation. This is supported by Villegas (1992) who examined effect of solid and liquid media on plant roots initiation. He reported that excessive callosity with 40% rooting was observed with the use of solid medium while liquid medium increased the rate of rooting to 80% and generated a notable reduction in callus mass. Rooting in *P. biglobosa* is rather difficult, according to Chevre (1985) who opined in that rooting is often more difficult on ligneous plants than herbaceous plants. Another study (Indieka et al., 2007; Werbrouck et al., 2016) explained that high concentration 2 mg/L of IBA could only produce few numbers of roots in the regenerated shoots of *Melia volvensii*, confirming further that rooting is really difficult in the tree species.

Conclusion

The present study assessed the effects of various concentrations of growth promoters (BAP and IBA) on the micropropagation of *Parkia biglobosa* using explants from the apical and axillary buds. The study concludes that induction and responses of the explant in term of shoot elongation and callus formation depends on the concentrations of the grow promoters, also, a high amount of IBA may favours root formation in the *in vitro* cultured buds.

Conflicting Interest

The authors declare no conflict of interest of whatever form on the study.

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